F Media (for culturing keratinocytes with feeders)

For 500ml:

338ml F12 media (Invitrogen 11765-054)
112ml 1X DMEM (high glucose) (Invitrogen 11965-084)
25ml FBS
5ml 100X adenine
5ml 100X cholera toxin
5ml 100X Insulin
5ml 100X Hydrocortisone
5ml Pen-Strep + amphotericin (Gibco)
OR
5ml Pen-Strep + 1ml Fungi-Zone

To complete F media (for feeding 24hrs after plating on feeder layer):

500ml F media
5ml 100X EGF

100X Adenine (Sigma A2786-5G)

- Dissolve 121mg adenine in 50ml 0.05N HCl by stirring for 1hr
- Filter sterilize and store at -20ºC in 10ml aliquots

100X Cholera toxin (EMD Biosciences 227035)

- Add 1.2ml sterile water to 1mg vial (making a 10uM solution)
- Dilute 50ul of 10uM solution into 50ml HBES containing 0.1% BSA
- Filter sterilize and store at 4 ºC in 10ml aliquots

100X Insulin (Sigma I6634-50MG)

- Dissolve 10mg in 20ml 0.05NHCl
- Filter sterilize with a pre-wet filter with FBS (or filter when making F media)

100X Hydrocortisone (Sigma H0888-1G)

- Dissolve 25mg in 5ml cold 100%EtOH (to make a 5mg/ml solution)
- Add 0.8ml of 5mg/ml solution to 100ml HBES with 5% FBS
- Filter sterilize and store at -20ºC in 10ml aliquots

100X EGF (Sigma E1257-0.1MG)

- Dissolve 100ug vial in 10ml sterile water
- Add 90ml HBES containing 0.1% BSA
- Filter sterilize and store at -20ºC in 10ml aliquots

HBES (Hepes-buffered Earles' Salts) (Sigma E7510, H0887)

- Add 50ml Earles' to less than 400ml d^2H_2O
- Dissolve 11g $NaHCO_3$
- Add 12.5ml 1M Hepes buffer
- Bring to 500ml and filter sterilize (can scale down volume accordingly)

Treatment of Biopsies

1. Feeders (optional)
- NIH/3T3 J2 cells irradiated at 6000 rad or treated chemically (mitomycin) if no irradiator is available
Frozen in 10% DMSO, 10% FCS-DMEM
Store in liquid nitrogen or -70ºC freezer

2. Plate feeders (optional)
(Note: thawed cells can also be added to trypsinized biopsy prior to centrifugation)
Plate a 6cm plate with feeders (2 drops per 6cm plate) in 10% FCS-DMEM (1% Pen-Strep)
Allow to adhere (>4hr, <48hr) prior to plating biopsy

3. Treat biopsies
1-2 biopsies collected in F-media w/o EGF in 15ml Falcon tube (can be kept up to 18hr at 4ºC before processing)

Let biopsy fall to the bottom of the tube and carefully aspirate media
Add 1ml trypsin
Let biopsy fall to bottom of the tube and carefully aspirate trypsin
Add 2ml trypsin and incubate 5-15min in 37ºC incubator
Inactivate trypsin by adding F media w/o EGF (optional: can add feeders at this point)
Centrifuge for 5min at 1200rpm
Carefully aspirate supernatant
Add 5ml F media w/o EGF and plate everything in a 6cm plate

24 hrs later discard 50% of media, careful to leave as much biopsy as possible
Replace with 5ml F media plus EGF

4. Culture
Change media 1 to 3 times per week
If needed (cells increase in size) add feeders every 1-2 weeks
In 1-2 weeks, keratinocyte colonies should form
In 2-4 weeks, the plate should be confluent
If few colonies are present at 14 days, passage the cells in a new 6cm plate plus feeders
When confluent, differentially trypsinize the cells (to remove feeders and any primary fibroblasts) and plate in two 6 well plates plus feeders, one 10cm plate plus feeders and freeze one to two 1ml vial(s)….
(note: for final/end point experiment, do not use feeders)